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Susceptibility of Tobacco Caterpillar, *Spodoptera litura* Fab. (Noctuidae : Lepidoptera) to certain Entomogenous Fungi

V. Deva Prasad, S. Jayaraj and R.J. Rabindra
Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore - 641 003

ABSTRACT

Five isolates of three entomogenous fungi were bioassayed for their infectivity to second instar larvae of *Spodoptera litura* Fab. by spraying the host larvae with the conidial suspension in the laboratory. Of them, *Beauveria bassiana* (Bals.) Vuill. (Bapatla isolate) was found to be the most virulent recording the lowest LC_{50} of 19.90×10^5 conidia ml^{-1}. The LC_{50} values of the Bangalore and New Delhi isolates of *B. bassiana*, *Paecilomyces fumosoroseus* (Wize) Brown and Smith and *P. farinosus* (Holm ex Gray) Brown and Smith ranged from 5.55×10^8 to 5.58×10^9 conidia ml^{-1}. Bioassay of second, third and fourth instar larvae of *S.litura* for their susceptibility to Bapatla isolate of *B. bassiana* showed that susceptibility decreased with increase in age of the larvae in terms of both LC_{50} and LT_{50}.

Key words: *Beauveria bassiana*, *Paecilomyces fumosoroseus*, *Paecilomyces farinosus*, *Spodoptera litura*, susceptibility

Species of *Spodoptera* are known to be susceptible to almost all the groups of entomopathogens like viruses, fungi, bacteria, protozoa and nematodes. Considerable studies have been made only with the nuclear polyhedrosis virus which effectively controlled *Spodoptera litura* Fab. on cotton, tobacco, banana, blackgram and cole crops (Jayaraj, 1986). Though seasonal outbreaks of diseases caused by entomogenous fungal species of *Aspergillus*, *Beauveria*, *Metarhizium* and *Nomuraea* have been reported in this pest on several crops in India (Ramamurthi *et al.*, 1967; Battu *et al.*, 1972; Rao and Phadke, 1977; Zaz and Kushwaha, 1983 and Siddaramaiah *et al.*, 1986), no detailed studies have been made so far to find out the scope of utilizing them in IPM strategies.

In the present study, three isolates of *Beauveria bassiana* (Bals.) Vuill, one isolate each of *Paecilomyces fumosoroseus* (Wize) Brown and Smith and *P. farinosus* (Holm ex Gray) Brown and Smith were bioassayed against second instar larvae of *S. litura* with a view to quantify entomopathogenic fungal spore load to achieve 50 per cent mortality of the host larvae for an understanding of the relative susceptibility of the pest to the pathogenic fungal species or isolates. Susceptibility of three larval instars of *S. litura* to a highly virulent strain of *B. bassiana* was also studied in terms of LC_{50} and LT_{50}.

MATERIALS AND METHODS

The culture of *S. litura* was raised from field collected larvae on a modified semisynthetic diet (Shorey and Hale, 1965) The larvae were reared in individual glass vials and routine surface sterilization of eggs and rearing containers with 10 per cent formaldehyde was carried out to prevent viral and fungal contaminations of the healthy stock. Neonate larvae were transferred to cotton plant (*Gossypium hirsutum* L.) grown in pots and reared through the first instar.

Isolates of *B. bassiana* were obtained from Indian Agricultural Research Institute, New Delhi (NDL), Indian Institute of Horticultural Research, Bangalore (BNG) and Agricultural Research Station, Bapatla (BPT). Isolates of *P. fumosoroseus* and *P. farinosus* were obtained from Kerala Forest Research Institute, Peechi and Indian Agricultural Research Institute, New Delhi respectively. Larvae were first inoculated with the different isolates and the fungi reisolated in pure form from the diseased cadavers showing typical mycosis. Then, the isolates were maintained by subculturing in Sabouraud dextrose agar enriched with yeast for *B. bassiana* and potato dextrose agar for *Paecilomyces* spp. at 25°C.

The conidia for the bioassay tests were harvested from 10-day- old cultures just before use by washing the surface of the plates using 75-100 ml of sterile distilled water containing 0.02 per cent Tween 80 (Rombach *et al.*,1986) The viability of the conidia was determined just prior to application as suggested by Gillespie (1986). Conidial suspensions of different concentrations ranging from 10^4

TABLE 1. Susceptibility of second instar larvae* of *S. litura* to the fungal isolates

Fungus	Probit analysis of dosage-mortality response			
	$Chi^2_{(3)}$	Regression equation	LC_{50} Conidia $ml^{-1} \times 10^5$	Fiducial limits (95%) $\times 10^5$
B. bassiana (NDL)	0.33	Y = 0.29101x + 2.41611	7571.40	157.54 - 36387.81
B. bassiana (BNG)	0.59	Y = 0.28586x + 2.50028	5553.47	126.71 - 24339.60
B. bassiana (BPT)	0.47	Y = 0.30685x + 3.0664	19.98	4.10 - 97.38
P. fumosoroseus	0.22	Y = 0.30695x + 2.24557	9412.01	200.98 - 44076.84
P. farinosus	0.34	Y = 0.29854x + 2.09022	55788.04	286.76 - 108549.98

* @ 180/assay

through 10^9 conidia ml^{-1} were standardized for each isolate after assessing the number of conidia in the suspension with an improved Neubauer haemocytometer. Newly moulted second instar larvae of *S. litura* were bioassayed for their susceptibility to the different fungal isolates. Ten larvae taken in a Petri plate lined by a filter paper were directly sprayed with 2 ml conidial suspension using a hand atomizer. Three such replicates were maintained for each concentration. Control insects received a spray of only 0.02 per cent Tween 80 in sterile distilled water. After air-drying, the treated larvae were carefully transferred to individual glass vials containing freshly prepared semisynthetic diet (without formalin) and incubated at 25 ± 2°C. During the incubation period, the relative humidity was maintained at more than 95 per cent. The larval mortality was recorded at 6 h intervals until eighth day of treatment. From the eighth day data, percentage of larval mortality due to observable mycosis was calculated.

In subsequent study, the highly virulent isolate of *B. bassiana* originally obtained from Bapatla was used to study the relative susceptibility of second, third and fourth instar larvae to the fungal pathogen following the bioassay procedure already described.

RESULTS AND DISCUSSION

The data on the dosage-mortality response of the second instar larvae to the different fungal isolates following direct application of the conidial suspension indicated a good fit of the observed and expected responses based on chi-square test (Table 1). The slopes (regression coefficients), in general, were very low with all the fungal isolates. The comparison of LC_{50} however, revealed the differential susceptibility of the pest to the fungal isolates. Of the five isolates bioassayed, BPT isolate of *B. bassiana* was the most virulent to the test larvae with the lowest LC_{50} value. There was a sharp increase in the values of LC_{50} in other isolates among which the order of virulence was BNG followed by NDL isolates of *B. bassiana*, *P fumosoroseus* and *P. farinosus*.

When second, third and fourth instar larvae of the test insect were bioassayed for their susceptibility to BPT isolate of *B. bassiana*, it was observed that the susceptibility to infection decreased with the age of the larvae. The LC_{50} of the fungal pathogen was lowest in the second instar larvae which increased as the stage of the larvae advanced (Table 2). The LT_{50} (at 10^7 conidia ml^{-1}) also increased with the age of the larvae indicating that older larvae were relatively more tolerant to the infection (Table 3).

Dose-dependent responses in all the bioassays were not pronounced as evidenced in lower slopes in the present study. Similar phenomenon of shallow dose-mortality responses seem to be typical for fungus-insect interactions according to earlier workers (Hall, 1980; Ignoffo *et al.*, 1982; Rombach

TABLE 2. Probit analysis of dosage-mortality responses of different larval instars of *S. litura* to *B. bassiana*

Instar*	$Chi^2_{(3)}$	Regression equation	LC_{50} (Conidia ml^{-1}) $\times 10^5$	Fiducial limits (95%) $\times 10^5$
II	0.30	Y = 0.30663x + 3.08977	16.98	3.49 - 82.50
III	0.90	Y = 0.34600x + 2.65922	58.23	12.35 - 274.58
IV	0.15	Y = 0.35091x + 2.41296	235.69	57.26 - 970.13

* @ 180/assay

TABLE 3. Probit analysis of time-mortality responses of different larval instars of *S. litura* to *B. bassiana*

Instar*	$Chi^2_{(3)}$	Regression equation	LT^*_{50} h	
II	0.35	Y = 3.32635x - 1.82759	112.87	97.23 - 131.02
III	1.07	Y = 4.30272x - 3.94661	120.03	104.86 - 137.39
IV	0.75	Y = 8.39851x - 12.77790	130.86	119.15 - 143.72

* at 10^7 conidia ml^{-1}

and Gillespie, 1988). The differential susceptibility of the pest to the different pathogenic fungal species or isolates in this study may be attributed to the inherent variations in the susceptibility of the host to the fungal pathogens. The biochemical interactions in the infection process which may be specific to host-pathogen interaction might have contributed to the differential susceptibility. Earlier, variations in susceptibility of *Trichoplusia* niHb. (Ignoffo *et al.*,1976) and *Heliothis* spp. (Ignoffo and Garcia, 1985) to different geographical isolates of *Normuraea rileyi* (Farlow) Samson have been reported. Maniania and Fargues (1984) observed wide variations in susceptibility of some noctuids to isolates of different entomogenous hyphomycetes.

In the study involving BPT isolate of *B. bassiana*, susceptibility of the host insect to infection decreased as larvae aged. Similar phenomenon was observed earlier in larval instars of certain noctuids bioassayed for their susceptibility to *B. bassiana*, *N. rileyi* and *P. fumosoroseus*. (Gardner and Noblet, 1978; Ignoffo *et al.*, 1978; Fargues and Rodriguez-Rueda, 1980). Chemical constituents vary as the larvae advance in age resulting in progressive hardening of the cuticle and increased humoral defense mechanisms to the microbial infections (Boman, 1981). Higher susceptibility of younger instars to the fungal infection as observed in the present study is a critical factor as pests controlled only in the early stages are less likely to cause economic injury to the crop plants.

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